Fat-specific satiety in humans for fat high in linoleic acid vs fat high in oleic acid.

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Original Communication
Fat-specific satiety in humans for fat high in linoleic acid *vs* fat high in oleic acid

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Objective: To investigate the effect of 2 week use of oils high in linoleic (LA), γ-linolenic (GLA) and oleic acid (OA) on energy intake (EI), macronutrient composition, parameters of appetite and taste perception.
Design: A randomized placebo-controlled 2-week treatment, followed by a test day.
Setting: Two-week treatments, daily life; test day, laboratory restaurant.
Subjects: Eight overweight men and eight overweight women (body mass index 27.4± 1.5 kg/m^2).
Interventions: Three 2 week treatments, in which subjects replaced their habitual fat products by three different oils (relatively) high in LA (66.8%), GLA (20.2%) or OA (79.6%, placebo). The wash-out periods were 2 weeks. Before each intervention period, taste perception and sensory specific satiety was tested.
Results: Even though energy intake was higher during dinner, subjects ate relatively less fat with LA (45.0±9.4 E%, $P<0.05$) than with OA (48.3±8.3 E%). Subjects did not distinguish the oils with the different fatty acids from each other. There was no relation between satiety or fat-specific satiety and taste characterization without as well as with sucrose. Although no differences were seen for the AUC of the appetite profile, at 15:00 h subjects were less satiated with LA (46.1±6.2 mm, $P<0.05$) or GLA (45.1±5.8 mm, $P<0.01$) than after treatment OA (62.5±4.8 mm). 24 h EI on the test day was 7.6–8.0 MJ and did not differ between treatments.
Conclusions: Fat-specific satiety during dinner with LA *vs* OA was shown after a 2-week treatment of each oil, but no change in general satiety. Fat specific satiety was not related to taste perception or characterization of the oils.
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Descriptors: macronutrient composition; macronutrient specific satiety; fatty acids; appetite; taste perception
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Introduction

It is a common knowledge that the prevalence of obesity has increased in the United States (Kuczmarski *et al*, 1994), as well as in Europe (Seidell, 1995) during recent decades. In fact, it is becoming a serious health problem. Next to cardiovascular diseases (Kannel, 1997; Manson *et al*, 1990), obesity is related to other diseases or risk factors for diseases like hypertension, increased LDL and decreased HDL serum cholesterol concentrations, hyperlipidaemia and non-insulin dependent diabetes mellitus (Wing *et al*, 1989; Pi-Sunyer, 1993).

Diet-induced obesity is caused by long-term energy imbalance, in which energy intake is higher than energy expenditure. As an implication of excessive energy intake, fat intake is often associated with the development of obesity (Tucker & Kano, 1992; Romieu *et al*, 1988). Several studies have shown that fat intake is related to the fat mass of subjects (Dreon *et al*, 1988; Miller *et al*, 1990; Tremblay *et al*, 1989), which relationship was shown to be only a result of the relation between fat intake and energy intake (Westerterp *et al*, 1996). How does fat intake cause high energy intake and therefore contribute to weight gain and the development of obesity? Firstly, the hedonics of taste of foods might be important in food selection. Fat is palatable and therefore high-fat diets might be more tasty than low-fat diets and would promote consumption. A relationship between preference for fat and obesity is shown in that hedonic ratings for fats increase with increasing body mass (Drewnowski *et al*, 1985), and obese people

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have a stronger preference for high fat and sweet foods than lean subjects (Drewnowski, 1990). High-fat foods are energy dense because of its Atwater-factor, ie 37 kJ/g for fat, compared to 17 kJ/g for carbohydrates and proteins, which could affect energy intake as well (Drewnowski, 1998; Holt *et al*, 1995). The energy density of a food or diet, ie total metabolizable energy consumed with the macronutrients divided by the total weight of food and water consumed, is greatly determined by its fat content (Westerterp-Plantenga, 2001; Prentice, 1998; Drewnowski, 2000).

In addition, the poor ability of fat to induce short-term satiety might cause high energy intakes as well. High-fat foods seem to have a smaller effect on short-term satiety than foods high in carbohydrates (Lawton *et al*, 1993; Blundell *et al*, 1995; Stubbs *et al*, 1996; Rolls & Shide 1994). However, in the course of a day, macronutrient specific satiety for fat has been shown (Westerterp-Plantenga *et al*, 1996).

The high energy density of fat, together with the low ability of high-fat foods to induce short-term satiety may lead to passive overconsumption of fat and/or energy (Blundell & Stubbs, 1999; Lawton *et al*, 1993).

Fat intake has been mainly observed as a passive overconsumption facilitated by its hedonics, energy density and relatively low short-term satiety effect. However, little is known about the recognition of fat by the body. Recently, a relation between perception of fatty acids and dietary preference for fat has been shown in rats. Gilbertson *et al* (1997) demonstrated that free polyunsaturated fatty acids (PUFAs) inhibit delayed rectifying K^+ channels (DRK channels) in mammalian taste receptor cells, which in turn would lead to an increase in activity of the taste cells. Interestingly, the effects were only seen for *cis*-PUFAs (arachidonic, linoleic and linolenic acid), but not for the mono-unsaturated fatty acid oleic acid. Moreover, DRK channels in tongue tissue of OM rats with a preference for high-fat diets were less sensitive to the *cis*-PUFAs, than DRK channels of S5B rats. The latter rats prefer diets high in carbohydrates (Gilbertson *et al*, 1998). In addition, when placed on a high-fat diet, OM rats get rapidly obese, while the S5B rats reduce their food intake and remain lean. Thus, an inverse relation between fatty acid perception and fat preferences is seen in rats. Some evidence for a possible role of fat perception in humans has been shown by Tepper *et al* (Tepper, 1999; Tepper & Nurse, 1998). They showed a relationship between PROP-taster status and ability to discriminate between differences in fat concentrations in humans. Medium and supertasters were able to discriminate a 10% fat salad dressing from a 40% fat salad dressing.

Many studies have examined the effect of fat intake on satiety and/or food intake, compared to other macronutrients, without discriminating between different types of fat. Only a few studies have investigated the effects of saturation of fats on satiety. After a lunch high in oleic acid (55 E% (energy percent) of fat), energy intake during a buffet-dinner was higher than after a lunch high in linoleic or stearic/oleic acid (Lawton *et al*, 1997). From this study they concluded that mono-unsaturated fatty acids were less satiating than saturated or poly-unsaturated fatty acids. These results were partly confirmed by a study of French *et al* (French *et al*, 1998; French, 1999). They found that after intestinal infusions of linoleic, stearic and oleic acids, subsequent energy intake was reduced after linoleic acid infusion compared to saline.

Another study investigating the effect of fatty acids on body weight was conducted by Phinney *et al* (1998). They observed a reduced weight regain after weight loss by γ-linolenate. After weight loss, subjects who consumed 5 g of borage oil (high in γ-linolenic acid) regained less weight than subjects who used 5 g of olive oil (high in oleic acid) per day during a 1 y period.

The aim of the present study was to test the hypothesis that subjects were able to discriminate between different oils that were high in linoleic, γ-linolenic or oleic (placebo) acid. Subsequently, we assessed whether a diet high in linoleic or γ-linolenic acid was more effective in reducing energy or specific macronutrient intakes and inducing satiety compared to a diet high in oleic acid. Finally, we tested the hypothesis that in humans taste perception and satiety effects of oils with different fatty acids, ie linoleic and γ-linolenic *vs* oleic acid, are related.

Methods

Subjects

Forty-two subjects were recruited by advertisements in local newspapers. Twenty-six subjects (12 men and 14 women, body mass index (BMI) 25–30 kg/m^2, age 20–55 y) were selected for the experiment. For selection, subjects had to be healthy and at least 3 months weight stable prior to the study. They did not use any medication known to affect bodyweight and/or appetite, were non-smokers and only moderate alcohol-users. Eight overweight men and eight overweight women completed the study. Ten subjects dropped out for several reasons; three subjects for illness not related to the treatment and seven subjects because of the frequency of laboratory visits. Table 1 shows the baseline characteristics of the 16 sub-

Table 1 Baseline characteristics of the subjects ($n = 16$)

	All subjects (n = 16)	*Men (n = 8)*	*Women (n = 8)*
Age (y)	41.6 ± 8.6	41.9 ± 6.9	41.4 ± 10.5
BMI[a] (kg/m^2)	27.4 ± 1.5	27.8 ± 1.6	26.9 ± 1.5
HP[b]	14.8 ± 3.2	14.0 ± 1.9	15.5 ± 4.1
F1[c]	5.8 ± 3.6	4.5 ± 3.3	7.0 ± 3.5
F2[d]	6.1 ± 2.8	6.0 ± 3.6	6.1 ± 4.8
F3[e]	5.6 ± 3.6	5.5 ± 5.6	5.8 ± 5.2
PROP-taster[f]	9	3	6[g]

Mean ± s.d. [a]BMI, body mass index. [b]HP, Herman–Polivy restraint. [c]F1 (cognitive restraint) from the Three Factor Eating Questionnaire (TFEQ). [d]F2 (inhibition) from the TFEQ. [e]F3 (hunger) from the TFEQ. [f]PROP-tasters are supertasters and medium-tasters together. [g]$n = 7$.

jects. Body weight (after an overnight fast) was measured on a digital balance (SECA, model 707, Hamburg, Germany, weighing accuracy of 0.01 kg) while subjects were wearing underwear. Height was measured to the nearest 0.005 m. BMI (kg/m^2) was calculated as body weight (kg) divided by height (m) squared. Body weight was measured at the beginning and end of each intervention period. The degree of dietary restraint was determined by the Three Factor Eating Questionnaire (TFEQ; Stunkard & Messick, 1985) and by the Herman/Polivy restraint questionnaire (HP; Herman & Polivy, 1980).

PROP-taster status of the subjects was identified as follows. Since PROP-taster status does not influence intensity judgements for NaCl, NaCl serves as a standard against of which the PROP function can be compared. Five concentrations of NaCl (0.01 – 1 M) increasing in half-log steps and five concentrations of PROP (3.2×10^{-5}–3.2×10^{-3}M) increasing in half log steps were rated using a 150 mm visual analogue scale. Samples were tasted and expectorated. Subjects rinsed with water in between each sample. This procedure was used to generate suprathreshold taste intensity functions for the two compounds. When these functions are superimposed, the slope of the PROP curve appears much lower than the slope for the NaCl curve in non-tasters (NT). The PROP curve overlaps with the NaCl curve in medium tasters (MT). Supertasters (ST) have a steeper slope for PROP than for NaCl functions (Bartoshuk, *et al*, 1994). Because of missing data for one woman, data of PROP-taster status is presented for 15 subjects.

All subjects gave their written consent. The study was approved by the Medical Ethics Committee of Maastricht University.

Experimental design

The design of the study was a single blind, randomized placebo-controlled trial, which consisted of three 2-week treatments alternated by a 2 week wash-out period, to ensure that women were always in the same phase of their menstrual cycle. In the week before the start of each intervention period taste perception, taste characterization and sensory specific satiety of the oils was tested. At day 15 of each intervention period, the subjects came to the department for assessment of their 24 h food intake and appetite profile. Moreover, a blood sample was taken after an overnight fast.

The oil high in linoleic acid (LA) was 'Becel dieet olie' (Van den Berg, Unilever, Vlaardingen The Netherlands) and contained 66.8% LA. 'Borage oil' (Eurochem, Feinchemie GmbH, Munchen, Germany) was used as the oil containing a relatively high percentage of γ-linoleic acid (GLA, 20.2%). The placebo oil was 'Hozol oil' (Contined B.V., Bennekom, The Netherlands) which is high in oleic acid (OA, 79.6%). Oleic acid is the placebo fatty acid, since (Gilbertson *et al*, 1997, 1998) showed that oleic acid did not give any taste perception reaction.

Total fatty acid composition of the oils was determined by analyses on a gas chromatograph. Lipids were extracted according to the method of Folch. The oils were saponified and the fatty acids (FA) transmethylated to the corresponding methylesters (FAME) by reaction with acetyl chloride (Lepage & Roy, 1986). The FAMEs were separated and quantified by using a HP 5890 II gas chromatograph, fitted with a 50 m CP si188 capillary column with 0.25 mm i.d. and 0.12 µm film thickness (Chrompack®, Middelburg, The Netherlands). A standard fame mixture was used to identify the fatty acid methylesters by means of the retention times.

Taste perception and taste characterization of LA and of GLA rich oils were determined in comparison to the placebo oil, which was high in OA. Also 24 h food intake and the appetite profile were assessed with LA and GLA in comparison to OA. The methods are described below. The hedonic value was assessed during a pre-protocol phase, and did not differ between the oils (LA, 74.3 ± 23.2; GLA, 68.9 ± 36.5; and OA, 73.6 ± 30.7 mm/100 mm VAS).

Taste perception protocol

All taste perception tests were executed by the same experimenter, who took care that it was exactly the same procedure every time. The ability to discriminate between the different oils was tested using the triangle test. Subjects were offered three samples of oil: two alike, and one different. The question asked was 'which sample is odd'? This test is effective to determine if overall differences exist. Moreover, it can select subjects for ability to discriminate differences (Meilgaard *et al*, 1991). During such a test, the following procedure was used. Subjects rinsed their mouth with water, followed by chewing on a piece of white bread and rinsing their mouth again. The water and bread had to be expectorated. This procedure was followed after each sample. After they had rinsed their mouth, subjects tasted the oil sample. The sample also had to be expectorated. After a row of three samples, the subjects had to fill in a questionnaire with the question: 'which oil is odd to the other two?' After filling in the questionnaire, there was a resting period of 30 s before starting with the next session. Each time, two series of six trials were conducted; before the LA intervention, LA was tested against GLA (six trials) and OA (six trials). Before the GLA intervention, GLA was tested against LA and OA and before the OA intervention, OA was tested against LA and GLA.

The taste perception test was conducted without as well as with sucrose (9 g/l). Sucrose served as a stimulator of the taste cell.

Taste characterization protocol

In order to characterize the taste of the oils, the following test was executed, in a standardized form (CSO, Wageningen, The Netherlands). Subjects tasted and expectorated the oils. Before and after tasting each oil subjects rinsed their mouth with water followed by chewing on a piece of white bread and rinsing their mouth again. Subjects were asked: 'How neutral is the oil?' The subjects rated their characterization on a 100 mm visual analogue scale. The scale was

anchored from 'not neutral at all' on the left to 'extremely neutral' on the right.

24 h food intake and appetite profile protocol

During the 2 week intervention periods, subjects replaced their habitual fat products with one of the three different oils (LA, GLA or OA) each 2-week period. The order of use was at random. Subjects had to use 20 ml test oil for preparing their lunch and 25 ml for dinner during the intervention period. Furthermore, they were not allowed to use other fats (butter, margarine, low-fat margarine, oil etc) and they had to minimize the ingestion of products high in fat (cookies, cake, snacks etc). During the wash-out period, subjects consumed a self-selected diet. At day 1 of each intervention period, subjects were provided with the test oil. The oil had to be used for preparation of lunch and dinner, daily for a period of 2 weeks. At day 15 of each intervention period, subjects came to our department for assessment of their 24 h food intake and appetite profile. All subjects came in a fasted state (from 10.00 pm the evening before) and body weight was measured. After that, breakfast was consumed at home and it was the same for each test day. For lunch and dinner, subjects came to the department, where an *ad libitum* lunch/dinner was served. The lunch consisted of a pasta-salad (energy density 3.37 kJ/g, 23.7, 5.3 and 71.0% energy from protein, fat and carbohydrate, respectively) served with 20 ml test oil. For dinner, first 100 g of pasta and 100 g of bolognese-sauce (energy density 3.10 kJ/g, 26.7, 47.5 and 25.8% energy from protein, fat and carbohydrate, respectively) mixed with 15 ml test oil was offered. Furthermore a salad with 10 ml of oil was offered. This amount of food had to be eaten completely. After that, subjects could take more pasta and bolognese sauce *ad libitum* and in proportions chosen by themselves. Also, more salad was offered *ad libitum*. Subjects were allowed to eat a low-fat dessert *ad libitum*, which was either a low-fat fruit yogurt or a low-fat vanilla yogurt. Once subjects had chosen one kind of dessert, the same was offered on each test day. Snacks were offered *ad libitum* during the test day. The subjects were allowed to consume drinks and fruit during the test day. The experimenter recorded the amount of food eaten in the laboratory restaurant on this day. Subjects recorded the other foods consumed in a controlled food intake diary.

During the test day, the parameters of the appetite profile were measured with a number of questions using an anchored 100 mm visual analogue scale (VAS). The questions were the following: 'How hungry are you?'; 'How full are you?'; 'How satiated are you?'; 'How thirsty are you?'; (those questions were anchored with 'not at all'—'very'); and 'How much do you desire to eat?' (anchored 'not much'—'very much'). This took place at 10 fixed points in time: before and after breakfast, at mid-morning, before and after lunch, at mid-afternoon, before and after dinner, at mid-evening and before going to sleep (Westerterp-Plantenga *et al*, 1999).

At day 4 and 15 of the intervention period, a Dutch translation of the Three Factor Eating Questionnaire (TFEQ; Westerterp-Plantenga *et al*, 1999) was used to determine if there were changes in dietary restraint of the subjects during the intervention periods. Possible changes in dietary restraint were compared between the intervention periods. A mood questionnaire was filled in, as well at day 15 of each intervention period. Parameters of mood were measured with a number of questions using an anchored 100 mm VAS. The questions were the following: 'How relaxed are you?', 'How down are you?', 'How pleasant do you feel?', 'How angry are you?', 'How scared do you feel?', and 'How sad are you?' The VAS was anchored with 'not at all'—'very much'.

Sensory specific satiety

As a food is eaten to satiety, the pleasantness of taste of that food and the desire to eat it decrease more than of those foods which have not been eaten (Rolls, 1986). We hypothesized that if LA has a more pronounced taste effect than OA, sensory-specific satiety with LA would be stronger than with OA. We expressed sensory-specific satiety as a larger decrease of pleasantness of taste after consumption of the test oil, in comparison with the change in pleasantness of taste of the oils that were not eaten. To determine sensory-specific satiety the different oils (10 g) were offered with a cucumber salad (75 g). Before the test salad we determined the pleasantness of taste of each oil with small cups with cucumber (5 g) and oil (2 g). Each cup contained a different oil. The order of offer was at random. After the three small samples, the test salad was offered *ad libitum*. After the test salad, the three small cups with cucumber (5 g) and oil (2 g) were offered again. The order was the same as before the test salad.

While the subjects were consuming the small samples, they filled in a 100 mm anchored visual analogue scale, with the question: 'How pleasant is the taste now in your mouth?' This procedure was executed with one type of oil before the intervention period in which that oil was consumed.

Blood parameters

At day 15 of each intervention period, a fasting blood sample of 10 ml was obtained and mixed with EDTA to prevent clotting. Plasma was obtained by centrifugation (4°C, 3000 rpm, 10 min) and stored at −80°C until analysis of glucose by a hexokinase method (Roche Diagnostics, Hoffman-La Roche, Basel, Switzerland), triglycerides (GPO-trinder 337, Sigma), glycerol by a glycerolkinase-lipase method (Boehringer, Mannheim, Germany), β-hydroxybutyrate by the method of Moore *et al*, 1982) using a semi-automated centrifugal spectrophometer (Cobas Fara, Roche Diagnostics), and insulin with ELISA (Mercodia 10-1113-01).

Compliance
Fatty acid composition of plasma phospholipids was used to measure compliance. Lipids were extracted according to the method of Bligh and Dyer (1959). Aminopropyl bonded silica columns (500 mg/2.8 ml) were used to separate phospholipids from the total lipid extract (Kaluzny *et al*, 1985). The phospholipids were saponified and the fatty acids (FA) transmethylated to the corresponding methyl-esters (FAME) by reaction with 10% BF3 in methanol at 100°C for 1 h (Morrison & Smith, 1964). The FAMEs were separated and quantified by using a HP 5890 II gas chromatograph, fitted with a 50 m CP sil88 capillary column with 0.25 mm i.d. and 0.12 μm film thickness (Chrompack®, Middelburg, The Netherlands). A standard FAME mixture was used to identify the fatty acid methyl-esters by means of the retention times.

Statistical analysis
Data are presented as means and standard deviations. Data of plasma fatty acid composition of phospholipids are expressed as %wt/wt of total fatty acids.

The required number of correct answers in order to show significant differences in taste perception at the triangle test is five out of six (83.3%). This consists of 33.3% of giving the right answer by chance, plus at least 50% right of the remaining discrimination possibility (Meilgaard *et al*, 1991).

Possible differences in taste characterization between treatments were analysed with a repeated-measures analysis of variance (ANOVA). *Post hoc* analysis was done with the Scheffe *F*-test (Statview SE Graphics™). Difference in taste characterization of the oils without and with sucrose was tested with a paired *t*-test (Statview SE Graphics™).

Since the number of supertasters ($n=3$) was small in our study, we combined the group of medium-tasters and supertasters to one group, called 'tasters' ($n=9$). Possible differences in output parameters between tasters and non-tasters and between men and women were analyzed with unpaired *t*-test.

Possible differences in food intake, macronutrient composition, appetite profile, sensory-specific satiety and blood profile between treatments were analysed with a repeated-measures analysis of variance (ANOVA). *Post hoc* analysis was done with the Scheffe *F*-test (Statview SE Graphics™). The area under the curve of the appetite profile was calculated for 24 h interpolating the part that takes place at night.

Linear regression was used to analyze a possible relation between satiety and taste perception or satiety and taste characterization. The level of significance was set at $P<0.05$.

Results

Since no differences in gender were seen for taste characterization, appetite profile, energy intake, body weight change or sensory specific satiety, data for men and women are taken together. Only for taste perception did the results differ so these data are presented for men and women separately. No differences for PROP-taster status were seen for taste perception, taste characterization, appetite profile, energy intake, body weight change or sensory-specific satiety, so all data for PROP-tasters and non-tasters are taken together.

Compliance
Table 2 shows the fatty acid composition of plasma phospholipids. After 2 weeks of consumption of LA, the percentage of linoleic acid in plasma phospholipids was significantly higher than after a 2-week consumption of GLA or OA. Moreover, the percentage of γ-linolenic acid in phospholipids was raised after GLA consumption compared to LA or OA and the percentage of oleic acid in phospholipids was higher after OA consumption compared to LA or GLA.

Taste perception
Subjects did not distinguish the different oils from each other when the oils were presented without sucrose, nor with sucrose. On average, in 50% of the cases, the oils were detected correctly.

Women could distinguish GLA without sucrose from OA without sucrose better than men (Table 3). Moreover, women could distinguish LA with sucrose from OA with sucrose better than men. However, the women did not reach the discrimination level of 83.3% either.

Taste characterization
GLA (43.2±20.9 mm/100 mm VAS) was characterized as less neutral than OA (68.9±20.5 mm/100 mm VAS, $P<0.05$) and LA (63.2±24.2 mm/100 mm VAS, $P<0.05$).

Appetite profile
The area under the curve (AUC) for feelings of hunger, satiety, fullness, thirst and desire to eat did not differ between the three different treatments on the test day (Table 4). However, at 15:00 subjects were less satiated with LA (46.1±24.7 mm, $P<0.05$) or GLA (45.1±23.1 mm, $P<0.01$) compared to OA (62.5±19.2 mm) after a 2 week use of the same oils (Figure 1).

Table 2 Concentration of fatty acids in plasma phospholipids (%wt/wt of total fatty acids) of subjects ($n=7$) after 2 week consumption of oils high in linoleic acid (LA), γ-linolenic acid (GLA) or oleic acid (OA)

	LA	*GLA*	*OA*
Linoleic acid (%)	23.4±2.5	19.3±2.3[a]	20.1±1.0[a]
γ-Linolenic acid (%)	0.1±0.0[b]	0.3±0.1	0.04±0.0[b]
Oleic acid (%)	9.0±1.1[c]	9.0±2.3[c]	10.8±1.1

Mean±s.d. [a]Significantly different from LA ($P<0.005$). [b]Significantly different from GLA ($P<0.001$). [c]Significantly different from OA ($P<0.05$).

Table 3 Number of correct answers during the taste perception test of men ($n=8$) and women ($n=8$)

	LA vs *OA*	*GLA* vs *OA*	*LA* vs *GLA*
Without sucrose			
Men	2.4±0.8	3.1±1.1	2.4±1.0
Women	3.1±1.2	2.8±1.5	3.8±1.2[a]
With sucrose			
Men	2.4±0.5	3.4±1.7	3.2±1.6
Women	3.9±1.2[a]	3.1±1.0	4.8±1.2

Mean±s.d.
Since each combination was tested twice, the data given is the mean of two series of six trials. The oils, linoleic acid (LA), γ-linolenic acid (GLA) and oleic acid (OA) were tested without and with sucrose. In order to correctly identify the odd sample, subjects had to give five out of six correct answers.
[a]Significantly different between men and women ($P<0.05$).

Table 4 Area under the curve for parameters of appetite (measured 10 times during 24 h) at the test day after a 2 week consumption of oils high in linoleic acid (LA), γ-linolenic acid (GLA) and oleic acid (OA)

	LA	*GLA*	*OA*
Hunger	785±307	763±301	798±298
Fullness	891±355	917±411	944±410
Satiety	932±342	944±404	1091±280
Thirst	807±343	976±199	945±257
Desire to eat	821±320	736±292	860±263

Mean±s.d.

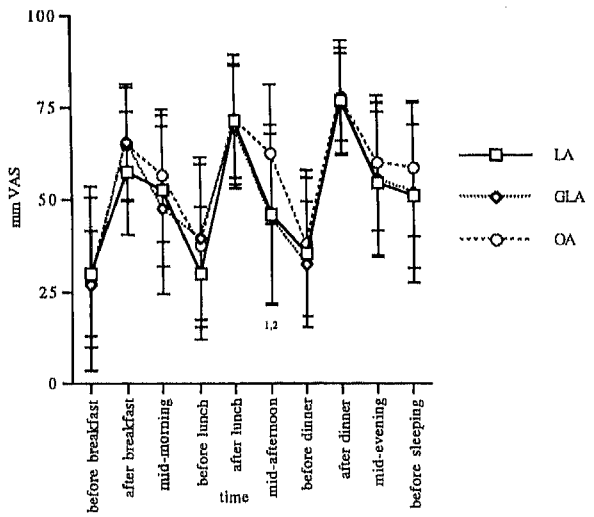

Figure 1 Satiety scores (mean±s.d. mm VAS) at the test days on which LA, GLA and OA were offered, after a two week use of the same oils. [1]LA significantly different from OA ($P<0.05$). [2]GLA significantly different from OA ($P<0.05$).

Energy intake
Total energy intake, measured at the test day did not differ between the three treatments (LA, 7.7±1.7; GLA, 8.0±2.6; and OA, 7.6±2.1 MJ/day). Differences in 24 h macronutrient composition were shown. Percentage energy from protein intake was higher on a test day with LA (12.3±1.8 E%) compared to OA (11.1±1.7 E%, $P<0.05$) after a 2 week use of the same oils. No differences were seen for carbohydrate and fat intakes (Table 5).

During dinner at the test day, energy intake was higher after a 2-week use of LA (3.0±0.8 MJ, $P<0.05$) as well as GLA (3.0±0.7 MJ, $P<0.05$) compared with OA (2.7±0.7 MJ). No differences in protein and carbohydrate intakes between LA, GLA and OA were seen. Moreover, fat intake at dinnertime after a 2 week consumption of LA (34.4±4.3) did not differ from OA (32.9±4.3). However, at the GLA condition, subjects ingested more fat than with the OA treatment (35.0±4.6 and 32.9±4.3 respectively, $P<0.05$; (Table 6). Differences in macronutrient composition (percentage of energy) during dinner between treatments were observed. Even though energy intake was higher, subjects ate relatively less fat with LA (45.0±9.4 E%, $P<0.05$) than with OA (48.3±8.3 E%). On the other hand, relative protein intake with LA (13.8±2.2 E%, $P<0.05$) and GLA (13.9±2.1 E%, $P=0.01$) was significantly higher than with OA (13.0±2.0 E%) after a 2 week use of the same oils (Table 7).

Body weight
Mean body weight loss during each intervention period was 0.7±1.0 kg ($P<0.05$). No differences were observed between the intervention periods. The cumulative weight loss of the intervention periods, was 2.2±2.2 kg. No

Table 5 Total energy and macronutrient (percentage of energy) intake at the test day after a 2 week consumption of oils high in linoleic acid (LA), γ-linolenic acid (GLA) and oleic acid (OA)

	LA	*GLA*	*OA*
Energy intake (MJ)	7.7±1.7	8.0±2.6	7.6±2.1
Protein	12.3±1.8*	11.8±1.7	11.1±1.7
Fat	35.1±3.7	36.1±4.7	36.2±5.2
Carbohydrate	51.6±3.0	50.8±4.4	50.8±4.3
Alcohol	0.9±2.5	1.3±3.2	1.9±3.6

Mean±s.d.
*$P<0.05$ compared to OA.

Table 6 Macronutrient intake (g) during dinner at the test day after a 2 week consumption of oils high in linoleic acid (LA), γ-linolenic acid (GLA) and oleic acid (OA)

	LA	*GLA*	*OA*
Protein	26.0±10.0	25.6±8.8	21.5±8.2
Fat	34.4±4.3	35.0±4.6*	32.9±4.3
Carbohydrate	78.2±32.1	73.9±26.1	64.2±24.9

Mean±s.d.
*$P<0.05$ compared to OA.

Table 7 Energy intake and macronutrient (percentage of energy) intake during dinner at the test day after a 2 week consumption of oils high in linoleic acid (LA), γ-linolenic acid (GLA) and oleic acid (OA)

	LA	*GLA*	*OA*
Energy intake (MJ)	3.0±0.8*	3.0±0.7*	2.7±0.7
Protein	13.8±2.2*	13.9±2.1**	13.0±2.0
Fat	45.0±9.4*	46.0±8.4	48.3±8.3
Carbohydrate	41.3±7.3	40.1±6.4	38.7±6.5

Mean±s.d.
*$P<0.05$ compared to OA. **$P=0.05$ compared to OA.

significant difference was seen for gender (men 2.7±3.5 and women 1.9±2.3 kg). Even though tasters lost more weight than non-tasters did, it did not reach the level of significance (3.1±3.1 and 1.2±1.9 kg, respectively; $P=0.09$).

Total weight loss over the intervention period, ie body weight at the beginning of the study minus body weight at the end, was 2.0±3.2. This did not differ for gender or PROP-status.

Dietary restraint
Scores on F1 (cognitive restraint) of the Three Factor Eating Questionnaire did not change during each treatment. Also, there were no differences between treatments (data not shown).

Mood
There were no differences in parameters of mood between each treatment (data not shown). For 'negative' parameters of mood (down, angry, scared, sad), all scores were under 35 mm measured on a 100 mm VAS. For 'positive' parameters of mood (relaxed, pleasant), all scores were above 50 mm.

Sensory specific satiety
The amount of test salad eaten was on average 30 g and did not differ between the different oil used (LA 29.0±18.0, GLA 28.6±20.1 and OA 30.7±22.8 g). The change in pleasantness of taste (from before to after the test salad) of the oil used in the test salad did not differ between LA, GLA and OA (−1.4±7.1, −6.5±15.0 and −4.9±15.4 mm VAS, respectively, $P>0.05$). Moreover, the change in pleasantness of taste of the oil used in the test salad (for example LA) did not differ from the change in pleasantness of taste of the oils not used in the test salad (OA and GLA) for LA, GLA and OA.

Blood parameters
There were no differences in glucose (5.10±0.15, 5.15±0.40 and 5.15±0.13 mmol/l) triglycerides (1586±429, 1362±242 and 1098±436 μmol/l), glycerol (108.4±11.0, 97.8±13.3 and 95.0±11.1 μmol/l), β-hydroxybutyrate (73.1±12.9, 89.3±25.6 and 75.7±24.2 μmol/l), and insulin (9.4±1.6, 8.2±1.3 and 8.7±1.1 mU/l) levels between the intervention periods (LA, GLA and OA, respectively).

Relation taste and satiety
Since no significant differences in taste perception of the oils was shown, but some significant differences in taste characterization of the oils was shown, we examined a possible relationship between taste characterization and satiety.

There was no relation between satiety (mm VAS at mid-afternoon and energy intake during dinner) and taste characterization for LA, GLA and OA ($r\leq 0.1$, $P>0.05$). Moreover, percentage energy from fat intake at dinner was not related to taste characterization for LA, GLA and OA ($r\leq 0.01$, $P>0.05$).

The difference in satiety as measured with difference in mm VAS at mid-afternoon and difference in energy intake at dinner between LA and OA was not related to the difference in taste characterization between these oils ($r=0.01$, $P>0.05$). Also, difference in relative fat intake during dinner and difference in taste characterization between LA and OA showed no relationship ($r<0.1$, $P>0.05$).

Discussion

After 2-week consumption of oils high in LA subjects ate relatively less fat during dinner at the test day, consisting of the same oil as consumed daily in the past 2 weeks, despite the higher energy intake during dinner compared to OA. Compliance for the 2 week consumption of the specific oils was confirmed by the fatty acid composition of the plasma phospholipids. The fat specific satiety was not related to taste perception ($r<0.1$, $P>0.05$), since there were no differences in taste perception between LA and OA. Even though taste characterization with sucrose differed between LA and OA, it did not show a relation with differences in relative fat intake during dinner. In rats, a relation between perception of fatty acids and dietary preference for fat has been shown. Gilbertson *et al* (1997, 1998) demonstrated that free polyunsaturated fatty acids (PUFAs) inhibit delayed rectifying K^+ channels (DRK channels) in taste receptor cells of Sprague–Dawley rats. Interestingly, the effects were only seen for *cis*-PUFAs (arachidonic, linoleic and linolenic acid). The same effects were demonstrated with tongue tissue of S5B/P1 rats, which have a preference for high carbohydrate diets. However, DRK channels of OM rats, which naturally prefer fats over carbohydrates, were not sensitive for *cis*-PUFAs (Gilbertson *et al*, 1998). In summary, these data suggest an inverse relation between dietary fat preferences and fatty acid sensitivity in rats. To the best of our knowledge, no data are available about the sensitivity of human tongue tissue to *cis*-PUFAs. Furthermore, our work was conducted with triglycerides in contrast with the studies of Gilbertson, who used free fatty acids (FFA) dissolved in ethanol. Triglycerides need to be

hydrolyzed by lipases in order to become free fatty acids. The presence of lingual lipase in humans is demonstrated (Hamosh *et al*, 1975; Hamosh & Burns, 1977) and activity is detected (Spielman *et al*, 1993); however the physiological role is uncertain. Different results might appear when one would use FFA.

The selective satiety for oil high in linoleic acid, that we observed, might be caused by different mechanisms, eg DRK channels that are selectively sensitive to *cis*-PUFAs in tongue tissue or in other parts of the digestive tract (Gilbertson *et al*, 1997, 1998). In addition, it has been shown that CCK release is higher after poly-unsaturated fatty acids (LA) than for mono-unsaturated (OA) and saturated fatty acids (Beardshall *et al*, 1989). This may contribute to the explanation of the fat-specific satiety that we observed in the situation with LA. The higher energy intake at dinner is likely to be due to the lower satiety at 15:00 h.

Recently, the effect of saturation of fatty acids on satiety was investigated by Lawton *et al* (1997) and French *et al* (French *et al*, 1998, French, 1999). Lawton *et al* gave subjects three lunches with 55% of energy from fat, in which the degree of saturation was varied, high in OA, high in LA or high in stearic acid. At dinner, subjects consumed significantly more after a lunch high in OA compared to the lunch high in LA. Moreover, post-ingestive ratings of fullness and motivation to eat were effected by the degree of saturation of the fatty acids. French *et al* administered intestinal infusions of LA, OA, stearic acid and saline. LA decreased subsequent food intake compared to saline. So, LA was shown to increase satiety in general and to suppress subsequent food intake. Our observations do not seem to be in line with their observations (Lawton *et al*, 1997; French *et al*, 1998; French, 1999) since we found that a meal with OA was more satiating than a meal with LA or GLA. However, Lawton *et al* offered a lunch that was very high in fat (55%, our study 45%), so their observation expressed as meal size might have been rather fat-specific as well. In our observations a meal with LA showed a higher fat-specific satiety, but a lower total satiety, expressed as meal size. It might be that the fat-specific satiety effect that we observed on the reduced fat intake with LA, after its use for 2 weeks, is generalized to meal size due to the higher dosage and emphasized by acute effects, in the study reported by Lawton *et al*.

Other differences in results found between the studies by Lawton, and by French, and those reported here might be caused by the period during which the oil was taken. In the study of Lawton *et al*, subjects consumed the oil only for one meal. In the study of French *et al*, subjects received the oils as intestinal infusions for 90 min, while in our study the oil has already been used for 2 weeks before the effects were tested. So, Lawton, as well as French were studying the acute effects, while in this study longer-term effects were observed. Also, the fact that the subjects had never tasted the oils before in the study of Lawton, can cause different effects compared to our study, in which the subjects had already used the oils for 2 weeks. Such a difference between acute and long-term observations might be due to possible adaptations to new fat consumption of the gastrointestinal tract (French *et al*, 1995; Cunningham *et al*, 1991; Shafat *et al*, 2000), although this does not have to change energy intakes. It is possible that short-term changes in appetite and/or energy intake may have been induced initially following commencement of treatment and these may have adapted towards pre-intervention levels at the time of testing.

The longer-term effect might have been an effect on long term sensory specific satiety with using the oils (Rolls & De Waal, 1985). However, we did check for short-term sensory-specific satiety, and we found no effects, nor differences between the different oils. Some indications for macronutrient specific satiety were shown by Westerterp-Plantenga *et al* (1996), who found a significant decrease in hedonic value for fat-foods after consumption of fat, a significantly increased desire for a different taste after consumption of carbohydrates, and a significant increase in satiety after consumption of protein all compared to a mixed diet. Johnson and Vickers (1993) also found some indications for macronutrient specific satiety, but not when it was expressed as decreases in food intakes.

No difference in taste perception and characterization, appetite profile and food intake or body weight was seen between PROP tasters and PROP non-tasters. In our study, seven out of 18 subjects (39%) were non-tasters. This percentage is comparable with the prevalence in a population: around 30% of the US population are non-tasters (Tepper, 1999). Furthermore, it is known that a greater percentage of women than men are tasters (Bartoshuk *et al*, 1994). In our population the 75% of the women were tasters, and 38% of men. Moreover all supertasters were women. It has been suggested that PROP-tasters could distinguish fats better than non-tasters (Tepper & Nurse, 1997). A recent study (Tepper, 1999; Tepper & Nurse, 1998) showed a relation between PROP-taster status and ability to discriminate between differences in fat concentrations in humans. Medium and super tasters rated the oiliness of a 40% fat dressing higher than a 10% fat salad dressing, while non-tasters did not. No differences in overall flavor and oiliness were seen between dressings within and between groups. However, medium and supertasters showed no preference for either dressing, while non-tasters preferred the 40% fat dressing. On the contrary, in a study by Drewnowski *et al* (1998), no relation was found between PROP-taster status and perceived intensity of creaminess, nor with hedonic value of different dairy products. Also, in our study, tasters (medium and supertasters together) as well as non-tasters were not able to discriminate between the different oils. However, although tasters gave a higher number of correct answers than non-tasters did, they did not significantly differ from each other. Several studies have been conducted to investigate a relationship between sensitivity to PROP and other tastes. At this moment it is not clear whether PROP-tasters are

better tasters in general. Recently, a new family of bitter-taste receptors has been discovered (Firestein, 2000). One taste cell contains many receptors, which are specific for different bitter compounds, ie caffeine, quinine and PROP. So, it might be that PROP-tasters are better tasters for bitter compounds in general.

In summary, oil high in linoleic acid showed after 2 weeks of consumption by humans, a selective satiety effect on fat intake by a relatively decreased percentage of energy as fat in the macronutrient composition, while it did not reduce total energy intake. This fat-specific satiety was not related to taste perception or taste characterization. PROP-taster status did not have an effect on energy and macronutrient intakes, and appetite profile. The hypothesis that humans may detect specific fatty acids in oils could not be supported by the data from this study, probably because we used triglycerides. For that, more observations on taste perception using free fatty acids are needed.

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